Nonclinical Pharmacology and Toxicology Review

BLA #:	97-0736
Product:	Zenapax® (dacliximab, humanized anti-Tac, ——— HAT, anti-Tac-H) concentrate for infusion (formulated as 5 mg/ml with 3.56 mg sodium phosphate monobasic monohydrate, 10.99 mg sodium phosphate dibasic heptahydrate, 4.6 mg sodium chloride, and 0.2 mg polysorbate 80, adjusted with hydrochloric acid or sodium hydroxide to pH 6.9).
Clinical indication:	Prevention of renal allograft rejection.
Sponsor:	Hoffmann-La Roche Inc.
Receipt date:	06-06-97
Assignment date:	06-19-97
Sections reviewed:	Nonclinical pharmacology and toxicology
# volumes:	19/264-25/264
Reviewer:	David M. Essayan, M.D.
Review completion date:	09-24-97

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ADME, absorption, distribution, metabolism, and excretion; AUC, area under the concentration-time curve; AUMC, area under the moment curve; CDR, complementarity-determining region; Cl_S, systemic clearance; C_{MAX}, peak concentration; ELISA, enzyme-linked immunosorbant assay; Fab, the antigen binding domain of the immunoglobulin molecule; Fc, the constant region of the immunoglobulin molecule; GVHD, graft vs. host disease; HAT, humanized anti-Tac; Ig, immunoglobulin; HuMikβ1, humanized anti-IL-2Rβ; IL-, interleukin; kD, kilodaltons; MAMA, monkey anti-mouse antibodies; MAHA, monkey anti-human antibodies; MAT, murine anti-Tac; Mikβ1, murine anti-IL-2Rβ; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; PK, pharmacokinetics; SCID (or scid), severe combined immunodeficiency syndrome; $t_{1/2}$, half life; Tac, T cell activation antigen, p55, the α chain of the IL-2 receptor (IL-2Rα), TCA, trichloroacetic acid; Vd_{SS}, volume of distribution at steady state.

Table of Contents

Abstract:

Zenapax® (dacliximab, Hoffmann-La Roche Inc.) is a humanized monoclonal antibody derived from a murine anti-human interleukin-2 receptor α chain monoclonal antibody; the final product exhibits strict species specificity for primate IL-2Rα. The binding of this Ab to its target interrupts IL-2/IL-2R interaction, resulting in inhibition of IL-2 induced T cell activation via the high-affinity IL-2 receptor; concurrent activation of ADCC by the Fc portion of the product may induce clearance of the reactive T cell clones and augment selective immunosuppression. This document reviews and summarizes the nonclinical pharmacology and toxicology data of the BLA application of Zenapax (97-0736) for use as an adjunct to standard immunosuppressive therapy in renal transplant recipients.

Introduction:

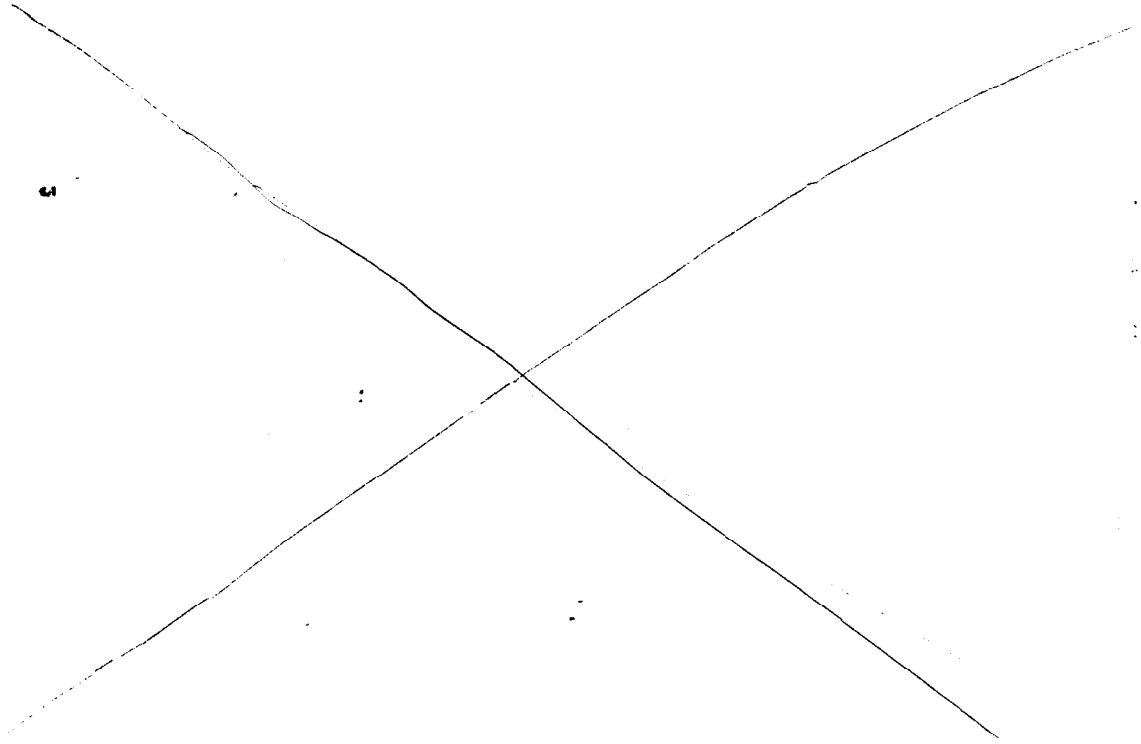
Acute episodes of renal allograft rejection occur in 20-50% of all recipients within the first 6 months after transplantation. These episodes lead to graft failure in 5-10% of primary recipients and an even greater proportion of secondary recipients; moreover, such rejection episodes and the measures taken to reverse them contribute significantly to the morbidity, mortality, and cost associated with renal transplantation.

In the forty years since the first renal transplant was performed, pre-, peri-, and post-surgical immunomodulation has been progressively refined in order to prevent rejection while minimizing complications associated with immunosuppression and general toxicity. One approach targets activation-induced antigens on immune cells; many currently used chemical immunomodulators (cyclosporin, tacrolimus) interrupt T cell activation by blocking IL-2 generation, thus abrogating ongoing immune responses. An alternative mechanism, utilized by the current product, blocks IL-2 signaling by inhibition of IL-2 receptor engagement.

Zenapax® (dacliximab, humanized anti-Tac. ——— HAT, anti-Tac-H) is a humanized monoclonal antibody (predicted molecular weight of 144 kD) derived from a murine anti-human p55 (Tac) monoclonal antibody (MAT) by grafting of the murine CDR sequences (antigen-binding site) to a prototype human IgG1 constant domain and the Eu myeloma antibody variable region framework; the final product, expressed in SP2/0 cells, retains less than 10% of the murine amino acid sequence and exhibits strict species specificity for primate IL-2Rα (the α chain of the IL-2 receptor). The binding of this Ab to its target is suggested to interrupt IL-2/IL-2R interaction, resulting in inhibition of IL-2 induced T cell activation via the high-affinity IL-2 receptor; concurrent activation of ADCC by the Fc portion of the product may induce clearance of the reactive T cell clones and augment selective immunosuppression. Zenapax is proposed for use as an adjunct to standard immunosuppressive therapy in renal transplant recipients.

In Vitro Pharmacology:

Four primary studies are reported in BLA 970736 to demonstrate that dacliximab i) retains high affinity for the Tac antigen, ii) exhibits strict species specificity for its antigen, iii) effectively block IL-2-mediated biologic responses *in vitro*, and iv) is capable of inducing ADCC against antigen-positive target cells.



Junghans *et al.* Anti-Tac-H, a humanized antibody to the interleukin 2 receptor with new features for immunotherapy in malignant and immune disorders. Cancer Res 50:1495, 1990.

1. HAT suppresses tetanus toxoid-driven T cell proliferation (IC_{50}=0.5-1 μg/ml).
2. HAT, but not MAT, is able to mediate CD16-dependent ADCC of susceptible targets; this activity is enhanced by IL-2 treatment of effector cells and by increased E/T ratios.
3. HAT is unable to mediate complement-dependent cytotoxicity.

Summary Comment-These 4 primary studies, in conjunction with other confirmatory data contained in this BLA submission, adequately support the sponsor's claims for this section.

Pharmacodynamics:

Thirteen primary studies are reported in BLA 970736 to demonstrate that HAT i) is effective in prolonging cardiac allograft survival in monkeys, ii) is effective in suppressing autoimmune disease in a variety of non-transplantation models, iii) is significantly less immunogenic than MAT, and iv) may be used safely in combination with selected additional immunosuppressive agents.

Brown *et al.* Anti-Tac-H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival. Proc Natl Acad Sci USA 88:2663, 1991.

1. Three groups of 5 cynomolgus monkeys underwent heterotopic cardiac allograft transplantation. Monkeys were either untreated or treated with either MAT (1 mg/kg) or HAT (1 mg/kg) beginning day -1 and continuing qOD until the time of rejection. Graft survival was used as the outcome measure.
2. Graft survival in the untreated, MAT, and HAT groups were 9.2±0.5, 14±2, and 20±0.6 days, respectively; $p<0.0025$ and <0.001 for the treatment groups vs. untreated, and ANOVA $p<0.02$ between the treatment groups.
3. Generation of MAMA (MAT) or MAHA (HAT) was also used as an outcome measure. 4/5 monkeys receiving MAT developed MAMA 1-10 days (mean 4) prior to allograft rejection; MAMA first appeared on days 6-15 (mean 11) after initiation of therapy and persisted for more than 17 months. The presence of MAMA was associated with rapid clearance of MAT and undetectable trough levels of MAT.
4. None of the monkeys receiving HAT developed MAHA prior to rejection. The peak MAHA titers were approximately 100-fold lower than the corresponding MAMA titers. Trough levels of HAT remained constant throughout the period of administration.

Tinubu *et al.* Humanized antibody directed to the IL-2 receptor β-chain prolongs primate cardiac allograft survival. J Immunol 153:4330, 1994.

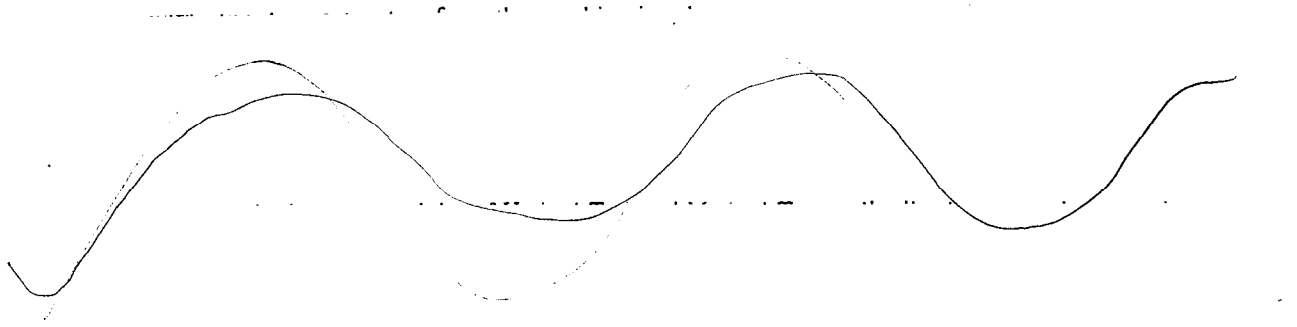
1. Six groups of 5-6 cynomolgus monkey underwent heterotopic cardiac allograft transplantation. Monkeys were either untreated or treated with HAT (1 mg/kg qOD starting on day -1 and continuing until rejection) alone or in combination with Mikβ1 or HuMikβ1 (1 mg/kg qOD starting on day -1 and continuing until rejection); Mikβ1 or HuMikβ1 were administered without HAT at the previously cited dose as controls.
2. Graft survival in the untreated, HAT, HuMikβ1, and HAT + HuMikβ1 groups were 8.2±0.4, 20±0.5, 27.8±4.2, and 19.4±1.5 days, respectively; $p<0.01$ for the treatment groups vs. untreated, but $p>0.3$ for the combination treatment vs. either single agent.
3. The addition of HuMikβ1 to HAT treatment increased the titer of and reduced the time to seroconversion to anti-HAT antibody production.

Reed *et al.* Prolongation of primate renal allograft survival by anti-Tac, an anti-human IL-2 receptor monoclonal antibody. Transplantation 47:55, 1989.

1. In the first study, 9 cynomolgus monkeys underwent heterotopic renal allograft transplantation with removal of both native kidneys. Monkeys were either untreated (n=5) or treated with MAT (2 mg/kg beginning day -1 and continuing qOD, n=4) until the time of death. In the second study, 9 additional cynomolgus monkeys underwent heterotopic renal allograft transplantation with removal of both native kidneys. Monkeys were either treated with cyclosporin alone (2 mg/kg qD, n=5) or cyclosporin with MAT (2 mg/kg beginning day -1 and continuing qOD, n=4) until the time of death. Overall survival and graft survival were used as outcome measures.
2. The mean number of days to rejection and death in the untreated group were 6 and 12, respectively; these values in the MAT group were 12 and 19, respectively ($p<0.05$). However, statistical analysis failed to document a significant difference between the group treated with cyclosporin alone and the group treated with cyclosporin and MAT.
3. Renal histology from the MAT group showed lower levels of infiltration by IL-2 receptor-bearing cells.
4. Serum MAMA appeared after 6-10 days of treatment in a cohort of 10 treated monkeys. Peak and trough MAT levels dropped to low or undetectable levels in all treated animals between days 7-10 of treatment.

Comment-The authors reported "low-dose cyclosporin effect[s] in some animals but not others, which obscure[s] a possible additive effect of anti-Tac."

THIS PAGE
WAS
DETERMINED
TO BE NOT
RELEASABLE



Hakimi et al. Reduced immunogenicity and improved pharmacokinetics of humanized anti-Tac in cynomolgus monkeys. J Immunol 147:1352, 1991.

1. Eight groups of 4 naïve cynomolgus monkeys underwent treatment with 0.05, 0.5, or 5.0 mg/kg/day of HAT or MAT vs. specific vehicle placebo for 14 days, followed by a single 5.0 mg/kg challenge dose of HAT or MAT on day 42. Immunogenicity was used as the outcome measure.
2. One monkey in the 5.0 mg/kg HAT treatment group had an apparent anaphylactoid response immediately following challenge on day 42, characterized by pupillary miosis, cyanosis, pale mucous membranes, reduced capillary refill, tachycardia, and respiratory distress associated with wet rales. No serologic or other assays were performed to confirm or refute the diagnosis of anaphylaxis. The animal responded well to treatment with epinephrine, dexamethasone, diphenhydramine, and saline hydration.
3. This reaction was not correlated to anti-HAT antibody titers; moreover, HAT-specific IgE could not be detected and total IgE remained unchanged in these animals.
4. Identical responses were seen in all four animals in the 0.05 mg/kg daily dose group of MAT rechallenged on day 42; rechallenge of the 0.5 and 5.0 mg/kg daily dose groups was not performed.
5. MAT-treatment resulted in earlier generation (days 9-15 vs. days 20-35) and higher titers (5- to 10-fold at comparable concentrations of antigen) of specific IgG than HAT-treatment. Interestingly, lower daily treatment doses were associated with greater degrees of primary response and anamnestic response upon rechallenge.
6. While the anti-MAT antibodies were a mixture of anti-isotype and anti-idiotype, the anti-HAT response was predominantly anti-idiotype. A subsequent study by Schneider *et al* (J Immunol 150:3086, 1993) indicated that the anti-idiotype response is directed against idiotopes composed wholly or in part of CDR regions H1, H2, and L3, and not to modified human V region framework.

Comment-It is speculated but not demonstrated that impurities present in this lot of material (purified by receptor affinity chromatography rather than the currently utilized ion exchange chromatography) could account for the anaphylactoid reaction to HAT. This remains the only study of HAT utilizing delayed rechallenge.

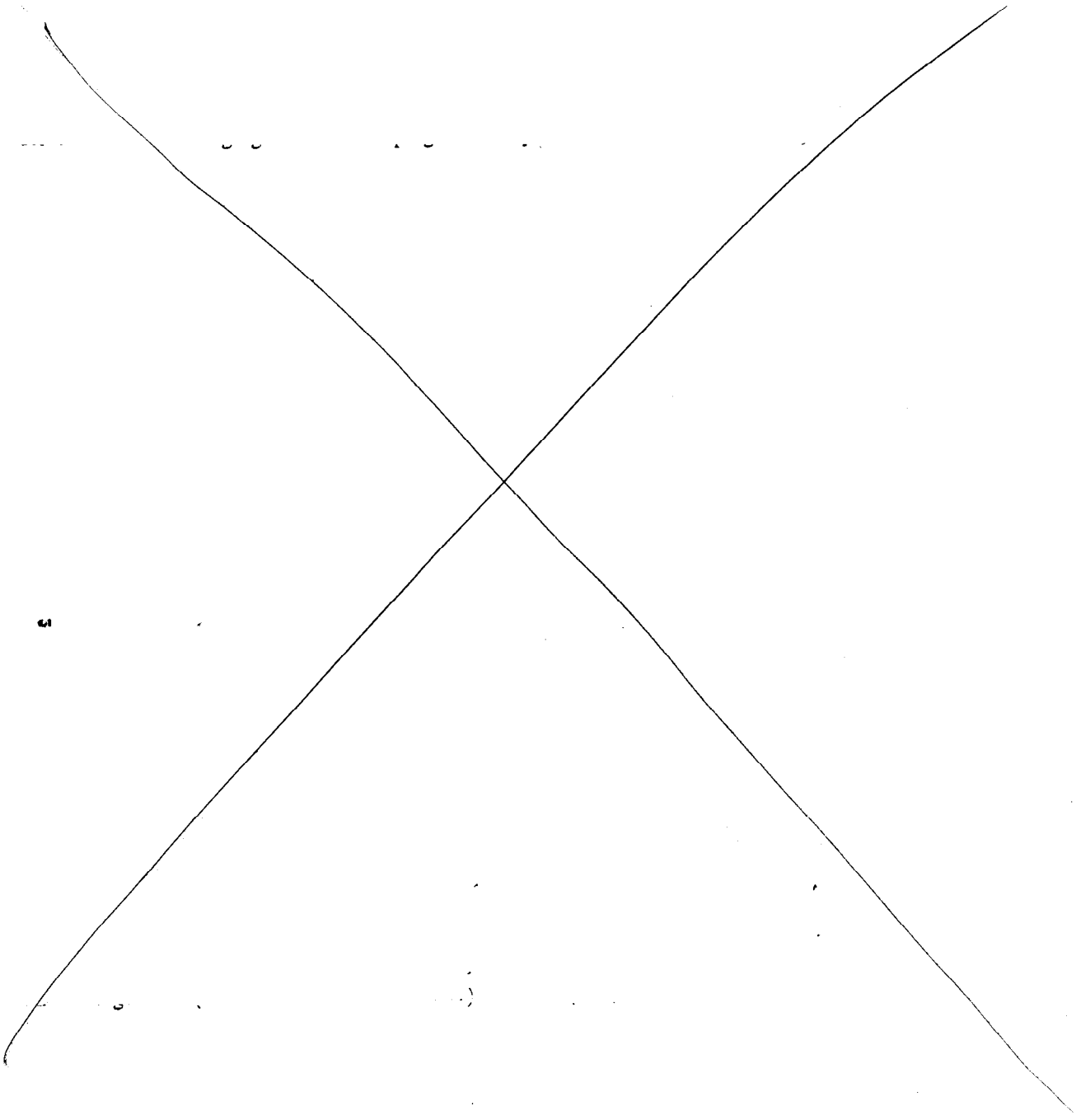
Group Number	Daily Dose Days 1 to 14, mg/kg	Challenge Dose Day 42, mg/kg	Response
1	Vehicle Control	HAT, 5.0	None
2	HAT, 0.05	HAT, 5.0	None
3	HAT, 0.5	HAT, 5.0	None
4	HAT, 5.0	HAT, 5.0	Anaphylaxis in 1/4
5	Vehicle Control	MAT, 5.0	None
6	MAT, 0.05	MAT, 5.0	Anaphylaxis in 4/4
7	MAT, 0.5	None [a]	—
8	MAT, 5.0	None [a]	—

[a] Monkeys not challenged with MAT because of anaphylaxis in Group 6.

<u>Summary Comment</u>-These 13 primary studies, in conjunction with other confirmatory data contained in this BLA submission, adequately support the sponsor's claims for this section.

Pharmacokinetics:

Seven primary studies are reported in BLA 970736 to support the clinical dosing regimen for HAT. All calculations are relative to the time of the first sample taken after intravenous dosing; AUC measurements are calculated by the trapezoidal rule, and extrapolated to infinity using the linear regression of the terminal slope. Terminal $t_{1/2}$ is calculated based on the linear regression of the terminal log-linear portion of the concentration curve. Cl_S and Vd_{SS} are derived from AUC and AUMC.



Hakimi et al. Reduced immunogenicity and improved pharmacokinetics of humanized anti-Tac in cynomolgus monkeys. J Immunol 147:1352, 1991.

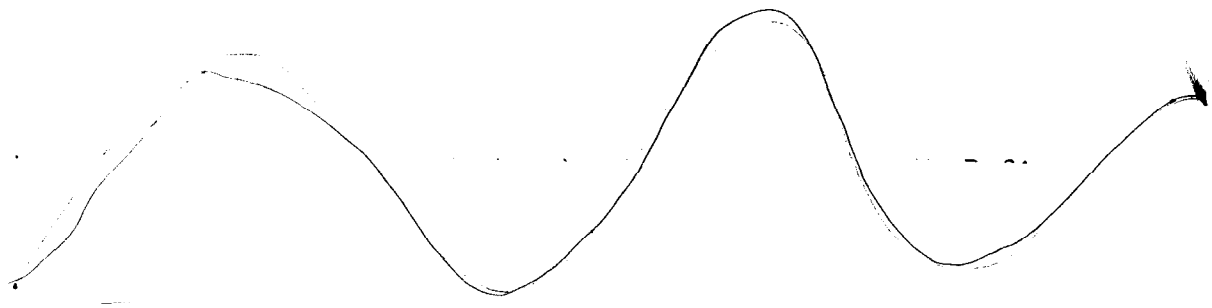
1. Eight groups of 4 naïve cynomolgus monkeys underwent treatment with 0.05, 0.5, or 5.0 mg/kg/day of HAT or MAT vs. specific vehicle placebo for 14 days, followed by a single 5.0 mg/kg challenge dose of HAT or MAT on day 42; serum samples were continued to day 50.
2. Comparative PK values for the single-dose administration of 5.0 mg/kg to vehicle control animals are as follows:

PK parameter	HAT	MAT
$AUC_{0\rightarrow\infty}$ (μg•hr/ml)	26657 (6237)	11442 (3563)
Cl_S ((ml/kg)/hr)	0.221 (0.114)	
Vd_{SS} (ml/kg)	52.5 (14.1)	
$t_{1/2}$ (hr, harmonic mean)	214 (59)	48 (9)

3. Following repeat administration, serum levels of HAT were measurable only in the 0.5 and 5.0 mg/kg dose groups; in general, trough levels increased over this 14 day time period. Comparative PK values for the single-dose administration of the 5.0 mg/kg challenge dose to HAT treated animals are as follows:

PK parameter	0.05	0.5	5.0
$AUC_{0\rightarrow\infty}$ (μg•hr/ml)	5852 (2682)	3275 (2290)	45402 (48510)
Cl_S ((ml/kg)/hr)	0.824 (0.668)	2.939 (3.044)	0.316 (0.363)
Vd_{SS} (ml/kg)	27.8 (7.2)	51.5 (13.2)	25.9 (3.8)
$t_{1/2}$ (hr, harmonic mean)	52	22	153

4. There is an inverse log-linear relationship between serum anti-HAT concentrations and PK parameters for all study groups.



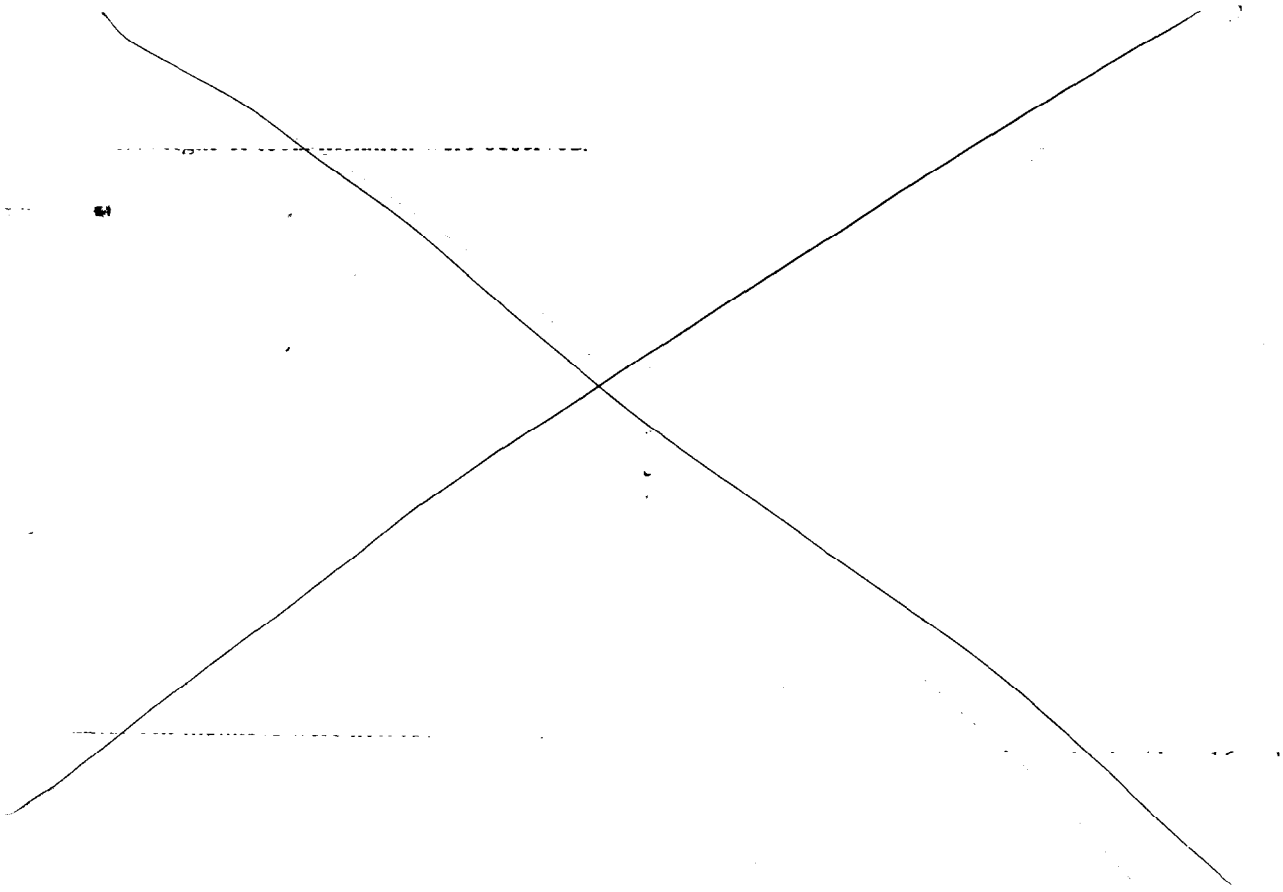
Brown *et al.* Anti-Tac-H, a humanized antibody to the interleukin 2 receptor, prolongs primate cardiac allograft survival. Proc Natl Acad Sci USA 88:2663, 1991.

1. Five cynomolgus monkeys received ^{131}I-labeled MAT or HAT; elimination was biphasic with terminal $t_{1/2}$ of 38±3 hours for MAT and 103±9 hours for HAT.
2. The daily catabolized fractions of HAT and MAT were 0.25±0.03 and 0.58±0.07, respectively.

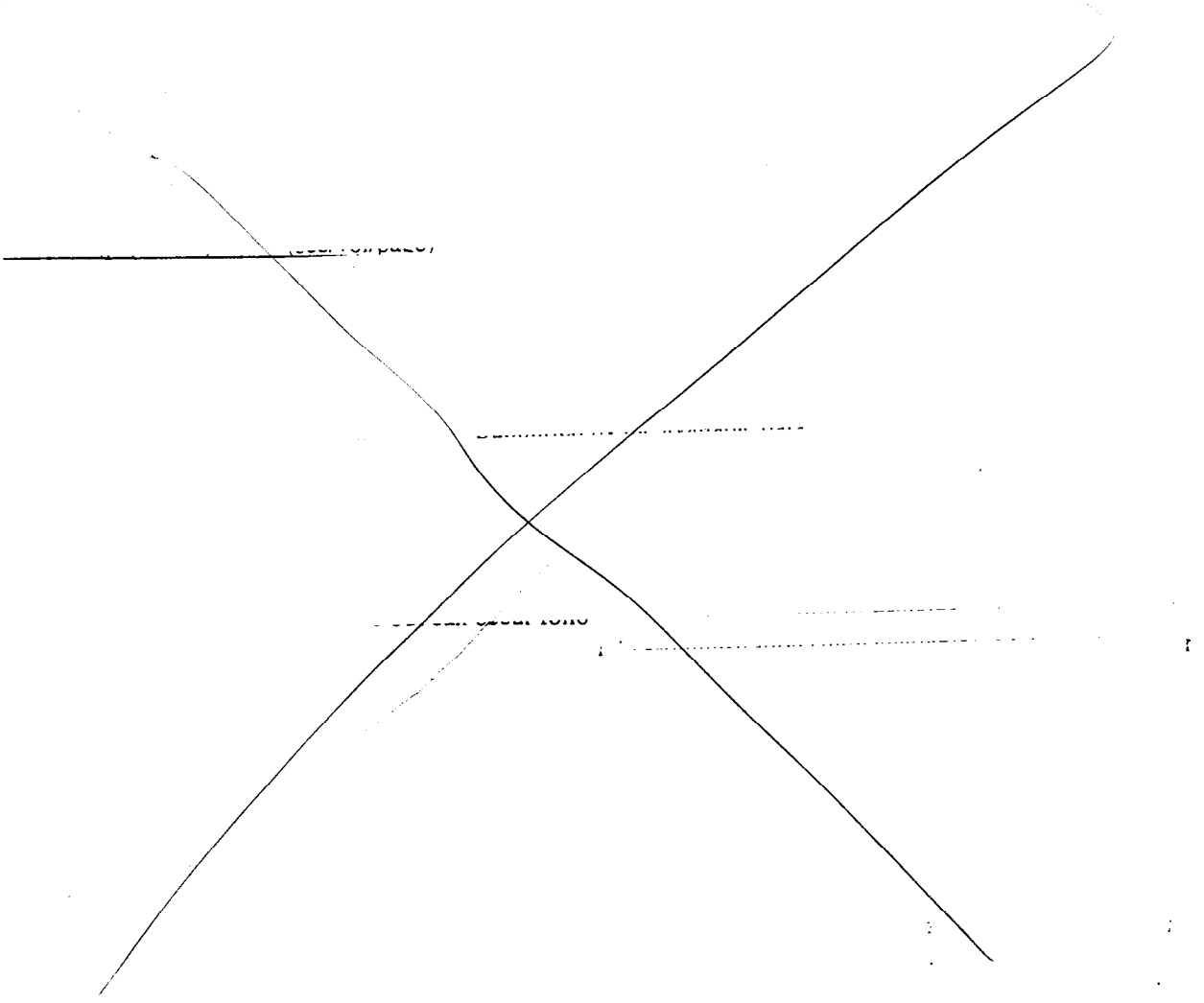
Summary Comment-These 7 primary studies, in conjunction with other confirmatory data contained in this BLA submission, adequately support the sponsor's claims for this section.

Preclinical Toxicology:

Eight primary studies are reported in BLA 970736 to demonstrate the safety of HAT by iv. infusion. Carcinogenicity, reproductive, and teratogenicity studies have not been performed with HAT.



Summary Comment-These 8 primary studies, in conjunction with other confirmatory data contained in this BLA submission, adequately support the sponsor's claims for this section.



Summary & Conclusions:

Zenapax® (dacliximab, humanized anti-Tac, ——— HAT, anti-Tac-H) is a humanized monoclonal antibody derived from a murine anti-human p55 monoclonal antibody, exhibiting strict species specificity for primate IL-2Rα. Zenapax is proposed for use as an adjunct to standard immunosuppressive therapy in renal transplant recipients.

This section of the review for BLA 97-0736 has summarized the available *in vitro* pharmacology, pharmacodynamics, pharmacokinetics, and preclinical toxicology data for this product. These studies suggest reasonable safety and efficacy in the preclinical models evaluated. ———

Recommendations:

David M. Essayan, M.D., Medical Officer.

Martin D. Green

M. David Green, Ph.D., Branch Chief, Clin. Pharm. Tox.